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**Animal feeding stuffs — Determination of  
castor oil seed husks — Microscope  
method**

*Aliments des animaux — Détermination des coques de graines de ricin —  
Méthode au microscope*

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 5061 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 10, *Animal feeding stuffs*.

This second edition cancels and replaces the first edition (ISO 5061:1983), of which it constitutes a minor revision. The specification for magnification has been changed.

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# Animal feeding stuffs — Determination of castor oil seed husks — Microscope method

## 1 Scope

This International Standard specifies a method for the determination of castor oil seed (*Ricinus communis*) husks in single and compound animal feeding stuffs and, in particular, in oilseed residues.

The limits of detection is 5 mg/kg.

The method requires final microscopical identification of the isolated husks. This last phase therefore requires a specialist who has had sufficient experience in this type of identification and who is experienced in microscopic techniques.

## 2 Principle

A test portion is boiled successively with nitric acid and sodium hydroxide solution. After washing, the residue is separated by decanting. The fragments of castor oil seed husks are dried, identified using a microscope, then weighed.

## 3 Reagents

Use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

**3.1 Nitric acid**, 10 % (by volume) solution.

**3.2 Sodium hydroxide**, 25 g/l solution.

## 4 Apparatus

Usual laboratory equipment and, in particular, the following.

**4.1 Stereomicroscope or binocular lens**, of total magnification  $\times 5$  to  $\times 50$ .

**4.2 Microscope and accessories**, of magnification  $\times 100$  to  $\times 1\,000$ .

**4.3 Oven**, capable of being maintained at  $103\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ .

**4.4 Nylon gauze**, of mesh size  $100\text{ }\mu\text{m}$ , resistant to dilute acids and alkalis.

**4.5 Sieve**, of aperture size 3 mm.

**4.6 Porcelain dish**, of capacity 1 000 ml to 2 000 ml.

**4.7 Measuring cylinder**, of capacity at least 1 000 ml.

**4.8 Flat-bottomed dish**, approximately  $140\text{ mm} \times 80\text{ mm}$ .

**4.9 Desiccator**.

#### 4.10 Analytical balance.

### 5 Sampling

It is important the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497.<sup>1)</sup>

### 6 Procedure

#### 6.1 Preparation of test sample

##### 6.1.1 Powdered feeding stuffs

Thoroughly mix the laboratory sample.

##### 6.1.2 Cakes or compressed feeding stuffs

Grind the laboratory sample coarsely so that it passes completely through the sieve (4.5). Mix well after sieving.

#### 6.2 Test portion

Weigh, to the nearest 0,1 g, about 100 g of the test sample into the porcelain dish (4.6).

#### 6.3 Determination

**6.3.1** Add 500 ml to 700 ml of the nitric acid (3.1). Bring to the boil, stirring continuously with a glass rod, and allow to boil for half a minute. Filter through the nylon gauze (4.4). Wash the residue with hot water and transfer back to the porcelain dish. Add 500 ml to 700 ml of the sodium hydroxide solution (3.2). Bring to the boil, stirring continuously with a glass rod, and allow to boil for half a minute. Transfer the suspension to the measuring cylinder (4.7) and fill the measuring cylinder with water.

**6.3.2** Pass a slight flow of water through the measuring cylinder by means of a glass tube immersed to a depth of two-thirds of the height of the measuring cylinder. Adjust the flow so that only the very finest particles remain in suspension and the husk fragments remain at the bottom. Continue this operation until the majority of the particles in suspension have been removed. Decant two-thirds of the liquid and filter the remainder through the nylon gauze (4.4).

**6.3.3** Transfer the residue to the flat-bottomed dish (4.8). Examine under the stereomicroscope or the binocular lens (4.1) and isolate the husk fragments on a white background using tweezers. Dry for 4 h in the oven (4.3) set at 103 °C. Allow to cool to ambient temperature in the desiccator and identify the fragments using the microscope (4.2) by comparing them with castor oil seed husks which have been subjected to the same treatment.

**6.3.4** Castor oil seed husks have a particular structure; the black or brown, acutely angled, husk fragments have a characteristic pitted surface which can be seen when examined under low magnification (see Figures 1 to 7).

Collect the husks and weigh them to the nearest 0,1 mg.

1) ISO 6497, *Animal feeding stuffs — Sampling*.

#### 6.4 Number of determinations

Carry out three determinations on test portions taken from the same test sample.

### 7 Expression of results

The castor oil seed husks content,  $w$ , expressed in milligrams per kilogram of the product as received, is equal to

$$w = m_1 \times 1,3 \times \frac{1\,000}{m_0}$$

where

$m_0$  is the mass, in grams, of the test portion;

$m_1$  is the mass, in milligrams, of dried castor oil seed husk fragments;

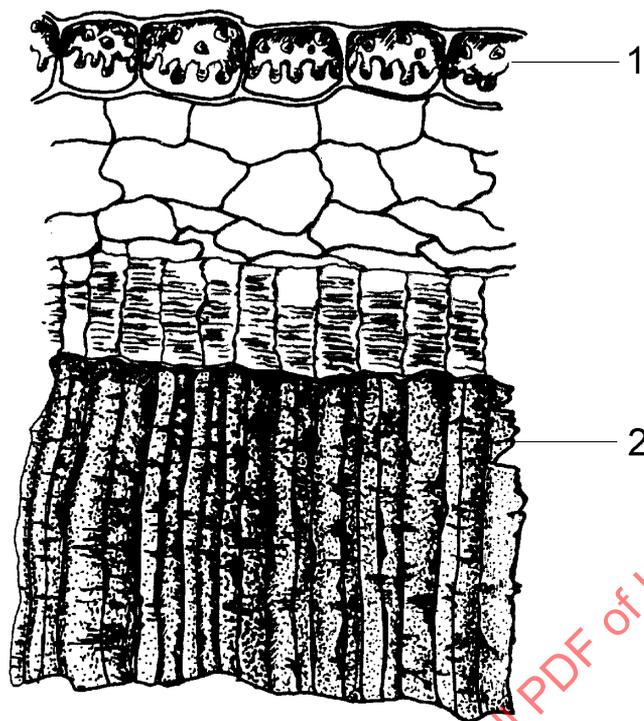
1,3 is a factor used to correct for the estimated loss of 30 % in the three steps of the analysis (6.3).

Express the result to the nearest unit as the arithmetic mean of the three determinations.

### 8 Test report

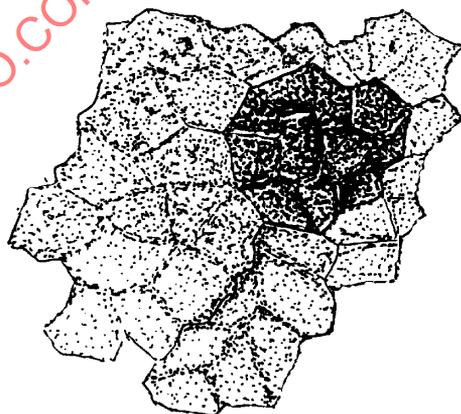
The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result;
- the test result obtained.



- Key**
- 1 Epidermis
  - 2 Fibres

**Figure 1 — *Ricinus communis* — Cross section of testa (magnification:  $\times 400$ )**



**Figure 2 — *Ricinus communis* — Epidermal cells of testa (magnification:  $\times 300$ )**

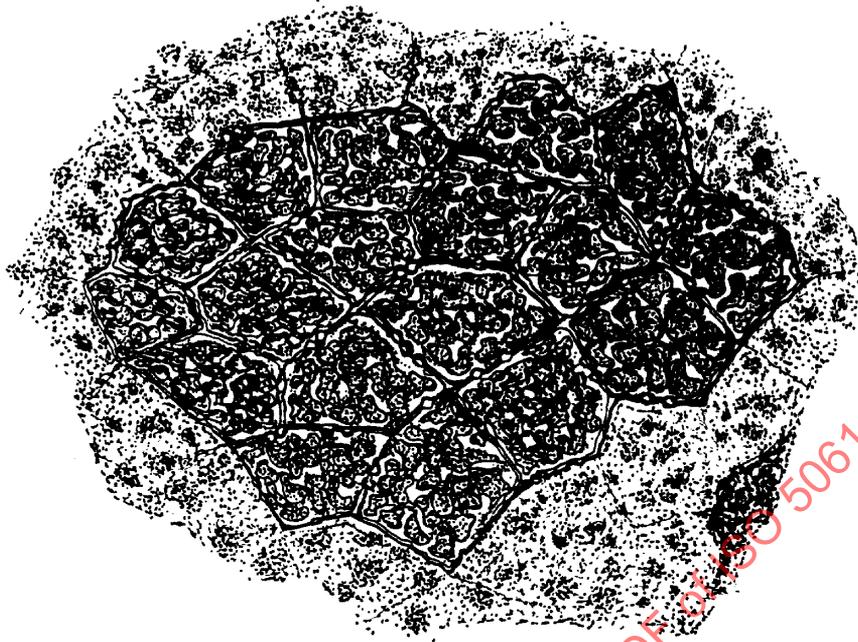


Figure 3 — *Ricinus communis* — Epidermal cells of testa (magnification:  $\times 500$ )

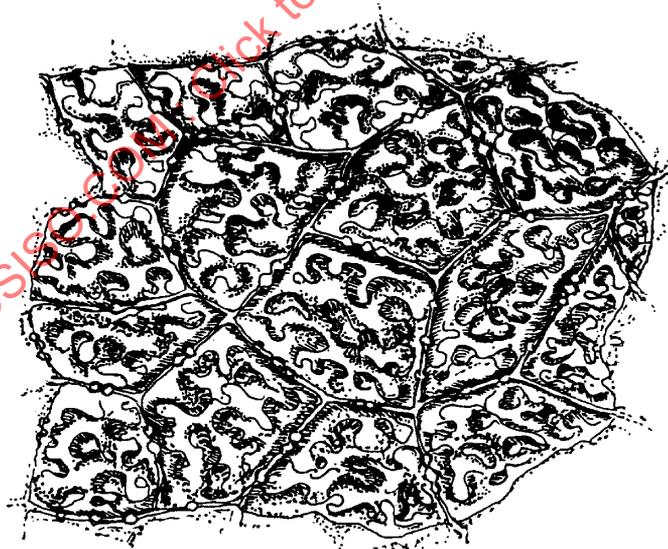


Figure 4 — *Ricinus communis* — Epidermal cells of testa (magnification:  $\times 800$ )